Presenter Abstracts

Plenary Session, Monday, 9:30 am - 10:50 am

Tools for Challenging DNA Samples

Novel STR Multiplexes with Reduced Size to Analyze Degraded DNA

Bruce R. McCord

The goal of this project is to develop novel STR multiplexes with reduced amplicon sizes for the analysis and detection of degraded and contaminated DNA. In such samples, DNA template can become highly fragmented due to bacterial, biochemical, or oxidative processes, and the possibility of finding an intact target sequence for PCR is greatly reduced. In this project researchers are developing and validating the use of STR Miniplexes[1]. These are CODIS STRs in which the primer sequences are moved as close as possible to the repeat region. A set of four miniplex kits have been developed covering 12 of the 13 STRs. In a series of studies with enzymatically degraded DNA researchers have demonstrated that reduced size STRs improve the success rate when degraded template is used[2]. Allele dropout is minimized and amplification efficiency improves. Investigators have also performed a concordance study of over 540 samples[3]. Of these samples, 16 discrepant samples were found, all but one of which occurred at vWA or D13. Several of these samples were sequenced, and the results show interesting effects due deletions or substitutions at the primer binding site.

Researchers have also applied these primers in the analysis of human skeletal remain samples (n = 31) that have been exposed to a variety of environmental conditions. These samples were obtained from the Forensic Anthropology Center (FAC) at the University of Tennessee in Knoxville and the Franklin County Coroner's Office in Columbus, Ohio. These samples provided a method to examine the efficiency of PCR amplification by the Miniplex primer sets using inhibited and degraded DNA templates. The amplification efficiency of these primer sets was then compared to the PowerPlex® 16 system.

Since many of the samples which will be analyzed using miniSTRs will also contain PCR inhibitors, the study has examined the effect of these substances on PCR amplification with Miniplex sets. The effects of these substances on the PCR reaction can vary from different levels of attenuation to complete inhibition. Researchers studied the effect of varying concentrations of PCR inhibitors using both Miniplexes and larger STR amplicons. In general the project found that while amplification efficiency improves with shorter STRs, the effect of PCR inhibition was the same. As a result of this work, various template cleanup steps were investigated, including spin columns, BSA and low melting agarose.

[Notes:]

Butler, J.; Shen, Y.; McCord, B. The Development of reduced size STR amplicons as tools for analysis of degraded DNA. *Journal of Forensic Sciences*, 2003**,** *48(5)*, 1054-1064.

Chung, D.; Drabek, J.; Opel, K.; Butler, J.; McCord, B., A study on the effects of degradation and template concentration on the amplification efficiency of the STR miniplex primer sets, *J. Forensic Sciences*, in press.

Drábek, J.; Chung, D.; Butler, J.; McCord, B. Concordance study between miniSTR assays and a commercial STR typing kit, *J. Forensic Sciences*, in press.

Assessment and In Vitro Repair of Damaged DNA Templates

Jack Ballantyne

DNA extracted from biological stains is often intractable to analysis. This may be due to a number of factors including a low copy number (LCN) of starting molecules, the presence of soluble inhibitors or damaged DNA templates. Remedies may be available to the forensic scientist to deal with LCN templates and soluble inhibitors but none presently exist for damaged DNA. In fact, knowledge of the biochemical nature and the extent of DNA damage in physiological stains is rudimentary at best. Also unknown is the point at which the damage inflicted upon a particular sample precludes the ability to obtain a genetic profile for purposes of identification. Therefore, the primary aims of this work were to first ascertain the types of DNA damage encountered in forensically relevant stains, correlating the occurrence of this damage with the partial or total loss of a genotype, and then to attempt the repair of the damage by means of *in vitro* DNA repair systems.

The initial focus of the work was the detection of damage caused by exogenous, environmental sources, primarily UV irradiation, but also factors such as heat and humidity. By incorporating various lesion specific enzymes, a set of assays, both PCR and gel-based, have been developed which describe the type and extent of damage inflicted upon DNA, both in a hydrated and dehydrated state. By dividing the UV spectrum into its component wavelengths, and combining each with various other conditions, the major causes of damage have been identified and their effects on genetic profiling assessed.

Increasing the Predictability and Success Rate of Skeletal Evidence Typing: Using Physical Characteristics of Bone as a Metric for DNA Quality and Quantity

David Foran

Obtaining genetic results from biological samples has become routine for forensic laboratories. In pristine condition, biological evidence will generate results that effectively identify the person who left it. In contrast, evidence that is compromised due to age, environment, and the like, begins to degrade, as will the DNA within it. The ability to effectively predict what genetic results are likely to be obtained from compromised biological evidence would help the DNA analyst choose the best technique for its analysis, potentially saving large amounts of time, effort, and materials.

The most common aged forensic material encountered is bone. In spite of this, little objective research has been conducted comparing the outward appearance of skeletal material (how weathered it is) and the quality or quantity of the DNA found within. The objective of the research presented here is to address just this, beginning with a set of skeletal remains obtained from the Voegtly Cemetery near Pittsburgh. The small cemetery was used for a short period of time, meaning the remains had little variation in age or environment, yet they contained a wide range of degradation levels. This study examines how and if skeletal weathering correlates with DNA quality and quantity. Specific bones within a skeleton, and localized soil conditions, were also assayed to see if either influences genetic results and bone degradation. Finally, bone samples from other locations were tested to deduce the generalization of the findings.

Developing a High-Yield DNA Isolation Method Using Matrix Protenase for Compromised Skeletal Samples

Richard C. Li

Mass fatality incidents have created a need to find new methods for identifying human remains. Bone tissue is often used for recovering DNA samples for the purpose of human identification. However, forensic science laboratories are challenged to determine identification when the evidence remaining is compromised skeletal fragments. The identification of partial DNA profiles or a failure to obtain a DNA profile has been reported when such samples were analyzed. It is believed that this is due to the degradation of genomic DNA in those samples. Also, in some cases the amount of sample is recovered in limited quantities, which makes DNA isolation more difficult. To recover an adequate quality and quantity of DNA template from such samples, a high-yield DNA isolation method is needed. This study is designed for developing a method to improve the yield of DNA isolation, thereby creating a higher success rate in generating genotype profiles for DNA analysis.

Plenary Session, Monday 11:10 am – 12:10 pm

Tools for Challenging DNA Samples Continued

Single Nucleotide Polymorphism (SNP) Detection in Highly Degraded DNA

George F. Sensabaugh, Jr.

DNA profiling is recognized as the "gold standard" for identifying human remains in mass disaster and human rights investigations. Despite the well-documented successes in making identifications in these cases, some samples contain either too little DNA or DNA that is too degraded for DNA profiling using current technology. The overall objective of the current research is the development of an alternative assay method for the analysis of biological samples containing highly degraded DNA. The assay detects single nucleotide polymorphisms (SNPs) using ligase mediated allele detection to generate a signal which is subsequently amplified by PCR. The assay scheme employs a solid phase platform to minimize two additional problems often encountered in the analysis of degraded biological remains: DNA loss during sample processing and inhibition of DNA amplification processes. Researchers have successfully demonstrated proof of principle on two solid-state platforms. Assay system parameters have been characterized and experiments on degraded DNA and DNA in the presence of inhibitors are ongoing.

Application of Mitochondrial DNA SNP Typing to Degraded Forensic Samples

Thomas J. Parsons

In this NIJ-funded project, whole mitochondrial DNA genome sequencing has permitted identification of eight multiplex panels of SNP sites over the entire mitochondrial DNA genome that can aid in increasing the resolving power of mitochondrial DNA typing in US Caucasians. Similar work is underway for identifying such sites for African Americans and Hispanics. Selection of appropriate Hispanic and African American mitochondrial DNA samples has required additional control region sequence databasing, which has been completed and will be

discussed. Meanwhile the eight Caucasian multiplexes have been designed as primer extension assays using SNaPShot reagents, and are in validation. Presenters will show the results of SNP typing on degraded casework material and present a missing persons case in which the SNP multiplexes were used to discriminate among individuals who otherwise could not be excluded.

Forensic Mitochondrial DNA Analysis and Mixture Separation by Denaturing High-Performance Liquid Chromatography

Phillip B. Danielson, Richard Kristinsson, Greggory S. LaBerge, Robert J. Shelton

Mitochondrial DNA analysis using denaturing high-performance liquid chromatography (DHPLC) is a novel approach to the detection of sequence variability in the mitochondrial D-loop. The technique relies on cross-hybridization of reference and questioned samples followed by high-resolution chromatographic separation of the resulting homo- and heteroduplices under partially denaturing conditions. The approach uses PCR primer sets that have been validated in forensic science for amplification of the HVI and HVII regions. The resulting chromatographic profile serves as a reproducible means of mitochondrial DNA sequence polymorphisms, heteroplasmy and multi-component mixtures. Pair-wise comparisons of hyper variable regions of human mitochondrial DNA from 96 unrelated human volunteers has demonstrated the ability to accurately identify sequence differences for each of four mitochondrial DNA amplicons in approximately seven minutes per sample. Thus, DHPLC analyses yield reproducible chromatographic profiles for maternally related individuals that are distinct from non-maternally related individuals possessing mitotypes that differ by at least one base pair.

The laborious nature, high cost and complexity of separating mitochondrial DNA mixtures has also been an obstacle to the broader use of mitochondrial DNA in forensics. Denaturing HPLC is being developed as a method for the efficient separation of DNA mixtures in preparation for DNA sequencing. The technology enables sequence-specific separation of mitochondrial DNA mixtures by high-resolution chromatography of cross-hybridized DNA. DHPLC can be used to isolate the individual components of natural (heteroplasmic) and situational (multi-contributor) mixtures. This is achieved without secondary amplification or excessive manipulation prior to DNA sequencing and will reduce the time and money required to obtain conclusive mitochondrial DNA results. DHPLC technology has the potential to benefit criminal and mass disaster investigations requiring timely analysis of mitochondrial DNA.

Working Lunch, Monday, 12:30 pm – 2:00 pm

Presenting Contested Mitochondrial DNA Evidence in the Courtroom

B. Michael Dann

Over the last decade, jury reform commissions, judges, and jury scholars have advocated the adoption of a variety of trial procedures to assist jurors in complex trials. These include reforms as prosaic as juror note taking through more controversial changes such allowing jurors to ask questions of witnesses or permitting them to discuss the case together during the trial. Although reform groups have endorsed many of these reforms for decades, their adoption has been slow. Lawyers in particular are wary about agreeing to trial reforms that might undermine their efforts on behalf of their clients. And there is only modest evidence about the impact of these reforms in the courtroom.

To add to this limited body of knowledge, Judge Dann recently collaborated with professor Valerie P. Hans, professor of psychology and law at the University of Delaware, and law professor David Kaye, Arizona State College of Law. The study, funded by the National Institute of Justice, compared the use of several jury reform techniques using a controlled mock jury approach. Mock juries composed of jury pool members watched a videotaped armed robbery trial, which included conflicting expert testimony about a relatively new technology for mitochondrial DNA analysis. Some mock juries simply watched the videotape and deliberated to a verdict. Others were permitted to take notes, ask questions about the scientific evidence, use a checklist, or refer to jury notebooks. The presentation discusses how the participants responded to the mitochondrial DNA evidence and how they employed the various reform techniques. Recommendations for jury trial procedures in cases with DNA evidence will be offered.

Plenary Session, Monday, 2:00 pm – 3:00 pm

Practitioner Presentations

Research in the Crime Laboratory

Eric Buel

When people think of scientific research, they primarily think of work being done in university settings or in biotechnology firms. While these may be the best settings for performing the basic research needed to lay the foundations for forensic applications, the crime laboratory itself is in a unique position to truly understand the types of R&D that can most benefit their own work. Crime laboratory staff also have an understanding of what it really takes to make a tool that can stand up to the scrutiny faced every day in the courtroom. Back in 2000, the laboratory decided to take on the research necessary to explore alternative ways to quantitate DNA. But how to do this, with limited resources and large case loads? This presentation discusses the evolution of NIJ-funded research at the crime laboratory, from a simple idea to automate slot blots, to the development, publication, and implementation of real-time PCR for DNA quantitation and more.

A Novel Approach to Expediting the Processing for No Suspect Sexual Assault Cases

Debra Figarelli

In many cases, DNA technology holds the key to solving violent crimes. However, limited personnel and financial resources have restricted the application of this powerful crime-fighting tool to "priority" cases for the Phoenix Police Department (PPD) Laboratory Services Bureau (LSB). Only those that are needed to meet court deadlines and the immediate needs of investigators in "high profile" investigations get analyzed. Faced with overwhelming requests for DNA analysis service, the PPD often cannot analyze no suspect cases.

The Phoenix Police Department has partnered with the Family Advocacy Center that provides services free of charge to victims of domestic violence and sexual assault offenses. The LSB provides the forensic nurse examiners with training and additional equipment that will enable them to assist in the identification of sexual assault cases that would be suitable for outsourcing. Forensic nurse examiners facilitate the screening of sexual assault evidence by the

LSB by performing a microscopic examination for the presence of spermatozoa at the time of the victim's examination. Electronic images of their findings will be captured and transmitted to the LSB Forensic Biology Section for review and reporting. Once the Forensic Biology Section has verified the spermatozoa identification, the sex assault kit can be marked for shipment to the outsourcing laboratory without any further processing by the LSB. This collaborative effort significantly reduces the amount of time spent by LSB examiners in screening the incoming sex assault kits and free them to concentrate their efforts on more complex screening in other cases.

A Home Invasion-Sexual Assault Unsolved to Solved

Ted Staples

As a result of NIJ's No Suspect DNA Backlog Reduction Program, a success story emerged out of Atlanta, Georgia. On November 29, 2003 at 11:15pm, a 32-year-old white female was home alone. While taking a shower she heard a loud noise towards the front of the residence. She stepped out of the shower, donned a towel and proceeded to investigate the noise. A man emerged from the hallway instructing her to return to the bathroom. He forced her to remove her towel and proceeded to sexually assault her. When he finished, he ordered her to give him all her money. He took $60, a laptop computer and fled. As she called 911 she observed her front door had been kicked open. Neighbors stayed with her until police arrived. No suspects were identified and no leads developed. The incident report ended with "This investigation continues."

No suspect DNA testing is performed at the Georgia Bureau of Investigation (GBI), but due to staffing shortages/backlogs, 'suspect' cases take priority. On implementation of the NIJ grant funding, however, a concerted effort began to target no-suspect DNA cases as the one described above. This case remained unsolved until March 4, 2004. A routine CODIS search resulted in an offender hit to DNA recovered from the rape kit. As has been observed in many instances now in Georgia since the offender database expansion to all convicted felons, the perpetrator identified in this case had not been incarcerated for a sex crime. This offender had been convicted of burglary. His criminal history was extensive in south and east Georgia involving theft, burglary, two counts of violation of motor vehicle law and escape. He served eight years for his last offense and was paroled in March 2003, just eight months before the attack. The agency is in process of closing out the case and a plea is pending.

This is one example of the violent crimes across the country that are being solved with the aid of NIJ's funding. With equipment and equipment upgrades supported by the No Suspect DNA Backlog Reduction Program, GBI has analyzed over 50 no suspect DNA cases.

Plenary Session, Monday, 3:20 pm – 4:20 pm

Practitioner Presentations Continued

Implementation of the No-Suspect Grant in a Small Laboratory

Cecelia A. Crouse, Julie Conover, Cathy J. Cothran, Karin A. Crenshaw, Dawn M. Hicks, Melanie M. McElroy, Amy B. McGuckian, Tara L. Sessa

This presentation delineates the successful implementation of the No-Suspect grant program and unforeseen issues that arose during this process. The Serology and DNA Section of

the Palm Beach County Sheriff's Office (PBSO) applied for and was granted a total of $950,000 for the 2002 and 2004 DNA No Suspect Backlog Reduction Grant. The goal of the project is to research every no-suspect case from over 37 Palm Beach County agencies and outsource DNA analysis on all viable no-suspect cases. This goal is being accomplished in three concurrent phases: (1) contract private investigation firm; (2) identify no-suspect casework evidence; and finally (3) review evidence, enter in CODIS, and prepare for judiciary.

Phase 1, "TopCop Investigations," involves collaboration between PBSO DNA section and ex-Detective Wayne Robinson and his firm. They have conducted four countywide "No-Suspect Seminars" to introduce the purpose and function of the grant, attended by all but one of county agencies. Mr. Robinson has helped the agencies research their case files. To date, he has reviewed over 17,000 case files from eight agencies, identified over 500 no-suspect cases, and requested submission of evidence from over 200 cases. Most cases were eliminated due to statute of limitations or lack of potential probative DNA evidence. For Phase 2 (post-identification of countywide grant-qualifying no-suspect case evidence), a submission protocol was enacted to track all incoming evidence and to coordinate submissions with the evidence custodian. Two analysts are assigned a month in which they prepare 15-20 cases for outsourcing. Evidence is screened for biological fluids or swabbed for epithelial cells, followed by documentation of the outsourced evidence. To date (January 2003 through March 2004), 15 batches or 377 forensic cases have been outsourced (each batch also usually contains a proficiency case). In Phase 3, on return of the DNA profiles obtained from the no-suspect casework evidence, there is a 100-percent review of all profiles to be entered into CODIS. The goal is to complete review and CODIS entry within a week of obtaining the vendor DNA information. Of the 317 cases that have been returned, there have currently been 211 profiles entered into CODIS (67%) and 79 hits (37%). An experienced ex-prosecutor creates "packets" for the State Attorney's Office (SAO) to summarize what is known about the hit (and no-hit) cases, so the SAO may then decide whether to prosecute or issue a "John Doe" warrant.

"Be All You Can Be": The Outsourcing Advantage

Stephanie Stoiloff

Outsourcing DNA cases has proven to be very worthwhile for the Miami-Dade Police Department (MDPD). The MDPD Crime Laboratory Bureau outsourced 1,324 cases as part of the FY 2001 No Suspect Case Backlog Grant. Analysis of no-suspect cases has led to the submission of evidence profiles to the Florida State DNA Database and resulted, not only in the identification of individual perpetrators, but in the linking of multiple cases to a serial offender. Knowledge that a serial rapist is preying on victims in a particular neighborhood, for example, enables detectives to sharpen their investigation and warn area residents.

The majority of cases sent were burglary (67%), robbery (12.7%) and sexual battery (12.7%). Data from 789 of these cases have been returned. Data from 699 cases has been reviewed and uploaded into the Combined DNA Index System (CODIS). From these 699 cases, 459 (66%) produced profiles suitable for entry into CODIS. Of these cases with DNA profiles, 51 percent of those profiles uploaded by MDPD into CODIS produced hits. Of these hits (46%) were connected to convicted offenders in the State and National DNA Databases. Also, 54 percent of DNA profiles produced hits to other cases (with suspects or evidence).

In addition to being able to outsource no-suspect casework, MDPD has also tracked the types of samples that have yielded DNA profiles, in order to determine how successful for DNA analysis a particular kind of sample is. For example, samples that have been tested ranged from

items such as zippers, socks, bottles and various burglary tools, to bloodstains from points of entry.

The data generated by the MDPD Crime Laboratory Bureau underlines the importance of processing the no-suspect cases that fill the evidence bins of laboratories nationwide. The State of Florida is progressing toward the collection of DNA from all felons in 2005. Together, the collection of convicted offender samples as well as the continued outsourcing of no suspect cases has allowed the MDPD to make a significant contribution to the police investigative effort by closing many cases and removing violent offenders from the streets of Miami-Dade County, Florida.

Interpretation of Human DNA Quantification Results from the Applied Biosystem Quantifiler™ Kits and PRISM® 7000 Sequence Detection System

Gary G. Shutler, James Currie, Jennifer Gauthier, Philip Hodge

The addition of new DNA quantification technology is needed to replace the current slot blot methods widely used in crime laboratories. Slot blot kits, such as the Applied Biosystem's Quantifiler™ method using the colorimetric development, do not have adequate sensitivity to use a negative result as an absolute predictor of whether an STR profile can be obtained or not. The outcomes of proceeding with PCR amplification of negative Quantiblot™ results can vary from obtaining a DNA profile, a DNA profile that may suffer stoichastic problems, or get no DNA profile. The recent acquisition of the Applied Biosystem's PRISM® 7000 Sequence Detection system and Quantifiler™ kits for DNA quantification through the NIJ No Suspect Backlog Reduction Program funding has provided an opportunity to address this problem. The Quantifiler™ kits and detection system provides the requisite sensitivity to compliment PCR-STR analysis. The preliminary internal validation results will be presented.

In addition to providing information on the amount of amplifiable DNA present in a DNA extract, the Quantifiler™ kits and detection system can also be used to predict problems with the presence of PCR inhibitors and degraded DNA. To assess this potential, bloodstains from 15 separate matrices are analyzed. They are; titanium, blue denim jeans, lead, steel, drift wood, spruce 2x4, pressure treated spruce 2x4, maple tree bark, ocean beach sand, garden sand, compost soil, rock, dried leaf, soiled dry leaf, and fresh grass clippings. DNA extracts were prepared from the bloodstains using a standard proteinase K digestion in buffer and organic extraction followed by a Centricon ® spin procedure. Previous work had identified PCR inhibitors present in the DNA extracts from five matrices. These were driftwood, 2x4 spruce, maple tree bark, ocean beach sand and compost rich soil. Inhibition was observed in the AmpFlSTR Profiler Plus™ kit results. Some of the inhibition results observed in the partial STR profiles resembled what would be expected for degraded DNA. When the inhibitory substance was removed by treatment with a QIAGEN QIAamp® mini column, the larger alleles were restored. The amplification plot results obtained from the Quantifiler™ kits for the IPC correctly predicted the inhibition problem for the two most potent inhibitors (soil and bark). Further results on this study will be presented.

Plenary Session, Tuesday, 9:20 am - 10:40 am

Innovative Tools for the Crime Laboratory - Presentations

Development of a High-Throughput Method to Isolate Sperm DNA in Rape Kits

Carll Ladd, Eric J. Carita, Alex Garvin, Henry C. Lee, Elaine M. Pagliaro, Timothy Palmbach

The large number of unprocessed sexual assault cases constitutes an ongoing concern for the forensic community. Many of these cases have sufficient numbers of sperm to generate DNA profiles that could be used to query the CODIS database and identify rape suspects. The standard method for purifying sperm from these swabs is to first resuspend all cells and to selectively digest the epithelial cells. The intact sperm are then separated from the contaminating solubilized DNA by centrifugation, careful removal of supernatant, and extensive washing of the sperm pellet, all steps that are difficult to automate. The project has developed a vacuum driven filtration method as an alternative approach for separating sperm from digested epithelial cells that are more easily automated in a 96-well format. Sperm are collected on two micron track-etch filters, while the epithelial cell DNA is collected in the filtrate. The filters are then washed, and the sperm DNA is solubilized with a reducing agent and collected in the filtrate. The goal of this project is to optimize and validate a faster, more effective, less labor intensive, and more cost-effective method to isolate sperm DNA from sexual assault samples to address the backlog of unprocessed biological evidence.

Innovative Hybridization DNA Typing for Forensic Applications

Winston C. H. Chen, Eric Buel, Bruce McCord

The objective of the project is to study the feasibility of making a PC device as platform for micro array DNA hybridization detection so that a reliable and inexpensive DNA detection instrumentation can be used for real time, in-situ DNA typing.

In the past, most micro array DNA hybridization detection has based the labeling of a DNA molecule on a single dye molecule. This approach replaces the single dye molecule tagging with a dye molecule impregnated particle. In this approach, each target DNA molecule is attached to a micro/nano particle that can contain billions of dye molecules. The detection is based on the fluorescence or light scattering of particles at the hybridization spots so that the detection sensitivity can be orders of magnitude better than the conventional single-dye molecule labeling method. Since different sizes of particles can be attached to a single DNA hybridization duplex, the detection sensitivity could potentially reach a few hybridized duplexes for each hybridization spot. Due to the high detection sensitivity, an expensive and delicate laser scanner micro array detector is no longer required. A simple digital camera for DNA, coupled with a light emission diode (LED) array, can be used for DNA hybridization detection. During the past year, researchers have successfully developed a prototype of Digital Camera Microarray Detector. It has been used to demonstrate hybridization detection for gene expression. Detection sensitivity has reached sub-femtomole region. It was also used to detect single nucleotide polymorphism (SNP). Future forensic applications will be actively pursued.

Gene Polymorphism and Human Pigmentation

Murray H. Brilliant

The overall goal of our research project is to determine with a high degree of accuracy, the pigmentation phenotype (hair, eye and skin color) of an individual subject from a forensic DNA sample. Human pigmentation is programmed genetically. Among the candidate genes mediating pigmentation variation are MC1R, ASP, P and MATP, each previously associated with mouse and/or human hypo pigmentation disorders that exhibit a spectrum of phenotypes. There are many additional gene candidates for human pigmentation variation based on mouse models. Polymorphism within these specific pigmentation genes may result in changes in the levels of expression of these genes or result in amino acid sequence variation (and function) of the corresponding proteins.

Researchers have recruited over 800 of a planned 1,000 subjects. The study has completed the genotyping of nearly 300 individuals for 117 individual polymorphisms in MC1R, ASP, P, MATP and several other genes including AP3, CHS, MYO5A, TYR, OA1, the HPS1-6 genes, plus five other genes.

In the first completed set of 300 individuals, 52 polymorphisms in a total of 16 genes show statistically significant associations with pigmentation phenotypes. Intriguingly, single polymorphisms in each of three genes can account for 66% of the phenotypic variation in skin pigmentation. This supports older genetic data suggesting that most of the variation in human skin pigmentation is associated with only three or four genes. Preliminary data suggest that the genetics of hair and eye color will be more complex. Thus, analyses of a human DNA sample can be a useful tool for forensic applications as a predictor of hair, eye and skin color.

A Genetic Database of Y-Chromosome Markers for Forensic Analyses

Michael F. Hammer, Alan J. Redd

The creation of a U.S. DNA database of Y-Chromosome markers is important for forensic scientists for three primary reasons. First, a series of short tandem repeats (STRs) will provide a valuable resource for estimating haplotype frequencies in cases of non-exclusion. Second, Y haplotype frequencies could be used in concert with autosomal STRs to obtain a joint-Y and autosomal-match probability. Third, the quantification of population structure within and among ethnic groups will guide the possible structure of future databases. That is, can investigators pool samples by ethnic group? Single-nucleotide-polymorphisms (SNPs) can be used to accurately quantify admixture among ethnic groups. The U.S. Y-Chromosome database consists of 30 short tandem repeats (STRs) and 50 single-nucleotide-polymorphisms (SNPs) in 30 U.S. populations. The Y-STRs include the U.S. core loci, the loci in commercially available kits, and many more highly variable STRs. The samples include individuals of European-American, African-American, Hispanic American, and Native American ancestry. Researchers find that the addition of a small number Y-STRs to the minimal haplotype greatly improves the ability to distinguish Y-Chromosomes in all tested U.S. population samples. Preliminary genetic structure analyses indicate strikingly similar patterns when comparing the Y-STR and Y-SNP data. Most of the genetic variation is found within populations (85% and 67% for the Y-STRs and Y-SNPs, respectively); a notable amount of variation is found between ethnic groups (14% and 32%, respectively); while only about 1% is found between populations within the same ethnic group. However, some Native American populations represent as exception to this general pattern. Although the genetic structure of U.S. populations reflects, in large part, their

ancestry, admixture varies dramatically across ethnic groups. Pooling populations into their ethnic group may be appropriate in many cases, although Native-American populations may require separate treatment. Researchers plan to make the database available to the forensic community in an online format.

Working Lunch, Tuesday, 11:30 am – 2:00 pm

Research Demonstrations and DNA Backlog Poster Session

A Demonstration of High-Throughput Methods for Reading and Typing LINEAR ARRAY™ Mitochondrial DNA Probe Panels

Cassandra D. Calloway

Researchers have developed and optimized a rapid method for the analysis of sequence variation in the HVI and HVII regions of the human mitochondrial genome utilizing the established technologies of PCR amplification and immobilized probe hybridization. This kit consists of two primer pairs for co-amplification of HVI and HVII regions and 33 probes immobilized in 31 lines for detection of sequence variation within ten segments of HVI and HVII. Using this rapid, informative assay, samples can be quickly screened to identify the most probative samples. Samples can be typed using a water bath or an automated typing instrument. Probe panels can be read manually by comparing to a reference guide or using a simple scanning software program. The remaining PCR product generated for the linear array assay can be used for sequence analysis if necessary. Additionally, the LINEAR ARRAYTM Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit consumes 50-75 percent less sample extract than sequence analysis because the HVI and HVII regions are amplified simultaneously rather than in two or four separate reactions.

Here, the presentation will demonstrate a simple scanning and interpretation software program that can be used to help with data analysis, and will demonstrate an automated typing procedure.

The Use of Amplified Fragment Length Polymorphism Analysis (AFLP) for Marijuana (Cannabis sativa) DNA Databasing

Eric Carita, Heather Miller Coyle, Carl Ladd, H.C. Lee, Elaine Pagliaro, Timothy Palmbach

Amplified Fragment Length Polymorphism (AFLP) analysis is a polymerase chain reaction (PCR) based DNA typing method in which PCR amplification of restriction endonuclease fragments are analyzed to individualize single source biological samples. The authors have recently completed state, national, and international AFLP marijuana (Cannabis sativa) databases composed of law enforcement seizure samples. The database has forensic significance in that it has the potential to identify and link clonally propagated marijuana plants with cultivators, distributors, and users as well as tracking certain "strains" that are distributed throughout the United States. In order to determine the genetic variability of marijuana and its statistical correlation, a marijuana population database was created from seizure samples provided by state and local authorities in Connecticut, Vermont, Florida, Iowa, Kentucky, Wyoming, Tennessee, West Virginia, Washington, California, Canada, and Taiwan. These

seizure samples were used to create state (110 sample), national (238 sample), and international (275 sample) databases. In addition, standard operating procedures are being completed for the microscopic examination of plant evidence and the identification and removal of foreign DNA (eg. mold) from plant evidentiary samples.

The database was created by using four selective primer sets (EcoRI-ACT FAM/MseI-CAA, EcoRI-ACT FAM/MseI-CAT, EcoRI-AAG JOE/MseI-CAT, and EcoRI-AAG JOE/MseI-CTA: A1, A4, F4, and F5 respectively) from the Applied Biosystem's AFLPTM Plant Mapping Kit. The amplified PCR was separated by gel electrophoresis on an (ABI) 377 DNA Sequencer. One hundred predetermined fragments are then scored using Genotyperâ analysis software (ABI) and converted to a binary code sequence that represents the samples genetic "profile." This combination of "1's" and "0's" is then imported into a database, which is used as a valuable search tool for identifying samples that are consistent with clonality. However, due to the fact that there is the possibility of two or more unrelated or half-sibling samples being represented by the same binary code, samples whose codes match are then superimposed upon each other in Genescan® (ABI) to determine whether any minor peaks outside the defined categories are detected. To assess the statistical significance of a match, the counting method, with a 95 percent confidence interval, is employed.

SpermPaints: Fluorescent Monoclonal Antibody Probes for Sperm Identification

John C. Herr, Linda Gilmer, Kenneth L. Klotz

SpermPaints is a mixture of fluorescent dye conjugated monoclonal antibodies that allows definitive identification of human sperm using fluorescence microscopy. SpermPaints contains: (1) a monoclonal antibody directed to the sperm head antigen, equatorial segment protein [ESP] and (2) a monoclonal antibody directed to the sperm flagellar antigen, calcium binding tyrosine phosphorylated protein [CABYR]. Together, this mixture of monoclonal antibodies stains the sperm head with a characteristic band pattern through its central region corresponding to the equatorial segment and stains the majority of the sperm tail corresponding to the principal segment.

SpermPaints is anticipated to have several advantages for identifying sperm in forensic casework. The ESP and CABYR target proteins are unique to sperm and testis, being differentiation antigens that arise during spermiogenesis. The property of these biomarkers results in the fluorescent signal from both monoclonal antibodies being clear, bright, and selective for sperm heads and tails with no cross reactivity to other tissues. The target antigens ESP and CABYR are stable: SpermPaints identified sperm in samples collected 1, 24, and 72 hours after coitus and from swabs that were stored at 4°C for two years. Significantly, the SpermPaints reagent: (1) identified sperm heads and tails when they were detached from one another; (2) identified sperm heads and tails that were masked by adherence to vaginal epithelial cells; and (3) identified sperm hidden within cellular debris. These performance characteristics of SpermPaints are anticipated to allow more rapid identification of sperm in microscopic fields and increase the number of conclusive identifications.

Demonstration of the GeneTrack DNA Forensics Chip System

Brian McKenna, Nils Goedecke

The Whitehead Institute, in collaboration with the state forensics laboratories, is developing an STR analysis system based on microfluidic chips. This system is designed to work in a portable crime lab and exceeds current commercial systems in data quality and analysis speed. It will evolve to an inexpensive "single-use," 16-lane, prepackaged chip, in which the samples may be retained. The current working prototype consists of a van-portable electrophoresis instrument that uses 11.5 cm 16-lane glass microchips to electrophorese conventional PCR samples in less than 25 minutes. Software analysis of raw electrophoresis data quickly produces an allelic profile that can be sent to CODIS or other DNA databases. The research is currently focused on performing protocol optimization, quality validation and robustness testing of the system. The presentation will demonstrate the system and reporting results from ongoing validation studies.

Prototype Polymeric Microfluidic DNA Analysis System for Forensic Analysis

Laurie E. Locascio, Michael Gaitan, Wyatt N. Vreeland

The presentation will describe the development of a prototype polymer-based microfluidic system for the rapid, multiplexed analysis of forensic DNA samples. The microfluidic system being developed for this project is a 16-channel system fabricated in thin sheets of polycarbonate. Devices are fabricated in polymeric materials in order to decrease costs and increase the future commercial potential of this technology. Since the device cost is low, polymer microfluidic systems can also be employed as single-use devices, preventing cross-contamination. In this presentation, researchers discuss results of DNA separations performed in polymer microfluidic systems emphasizing improvements in performance that have been achieved by altering device design, substrate material, and DNA polymer matrix used for the separation. Other critical aspects of this project that will be discussed include the development of new optical detection systems optimized for DNA detection in polymer devices (with P. Smith, N. Morgan, NIH); testing of new higher performance DNA separation polymer matrices (with A. Barron, Northwestern University); and development of software analysis tools and control systems for electronic interfacing and microfluidic switching (with T. Pohida, NIH). In this six month project, the key accomplishments have included optimization of DNA separations in a single polymer channel with samples amplified by PCR, fabrication of the 16-channel device, development of a sensitive and flexible miniaturized optical detection system, and development of software analysis tools. In the next six months, the research will focus much effort on the optimization of the 16-channel system.

Quantitation of Human DNA in Forensic Samples

Janice A. Nicklas, Eric Buel

This demonstration will have two parts running concurrently. The first part will consist of a step-by-step tutorial demonstrating quantitation of human DNA using real-time Alu PCR. An MS PowerPoint presentation gives a background explanation of the assay, demonstrating and comparing two real-time PCR instruments (the 72 tube Corbett Rotorgene 3000 and 96 well plate Stratagene MX3000P), showing how to setup real-time PCR, and performing software analysis of the data to determine human DNA concentration. There will also be handouts available with

protocols, reagent lists, and sample data. Individuals from the Vermont Forensic Laboratory and Stratagene will be available to answer questions about assay design, setup, or instrument acquisition. The second part of the demonstration will highlight the current NIJ-funded research projects of the Vermont Forensic Laboratory. This includes assays on gender identification, quick sample identification, and human DNA quantitation for short (mini) STRs. There will be explanation of the real-time probe based methods used for these assays (TaqMan® and molecular beacons) as well as presentation of data.

TrueAllele® System 3: Automated DNA Casework Interpretation

Mark W. Perlin

The human review of DNA evidence is a critical bottleneck that impedes DNA casework processing and contributes significantly to the DNA backlog. Moreover, complex DNA evidence (e.g., damaged DNA, mixed DNA samples, small DNA quantities) requires even greater care. It would be useful to have a computer resource that provides fast, accurate, understandable, reliable, objective and affordable DNA data review. For the past five years, Cybergenetics has been developing TrueAllele® System 3, an automated computer system for DNA casework interpretation.

Now in its 16th version, TrueAllele System 3 automatically solves challenging DNA casework problems, including degraded DNA, DNA mixtures, and low copy number. The TrueAllele system is particularly well suited for non-suspect DNA cases, since it infers unknown contributor genetic profiles without using suspect profiles. Scientific validation studies are showing that the TrueAllele computer system is at least as reliable as human review in many casework situations.

This computer demonstration will show TrueAllele® System 3 in operation. Particular emphasis will be placed on how the TrueAllele system translates complex casework data into understandable results. The presentation will include discussion and live computer interaction.

A Portable DNA Chip Instrument for Human Identification

Ronald Sosnowski

Different applications for human identification require different sample throughput rates and have different requirements for time to result. Field use would typically mean low throughput, but with a need for a quick turnaround time. Registering convicted felons, who are in custody, would not require as high a turnaround time, but throughput requirements would be higher.

Even though both applications require a DNA genotype as the end result, meeting the diverse throughput and turnaround requirements means that different solutions to customer needs must be considered. An analogous situation is the use of jumbo jets for high demand long distance routes versus turboprops for frequent commuter flights with varying demand.

This demonstration will display a prototype portable instrument designed for use with Nanogen's new active 400-pad chip. Originally designed for detection of bio-warfare agents, the original design has been refined to serve as a development platform for the NIJ-supported SNP identification panel. It has been used for assay development on the 400-pad chip while the higher throughput commercial instrument is being validated. A portable instrument will be on display and its features will be discussed. Comparisons with the commercial instrument will be pointed out, and the steps for doing DNA analysis will be demonstrated.

Quantitation of DNA by qPCR for Forensic DNA Typing: Single and Multiplex Assays for the Nuclear and Mitochondrial Genomes and the Y-Chromosome

Mark Timken, Martin Buoncristiani, Cristian Orrego, Katie Swango

The use of real-time quantitative PCR (qPCR) is proving to be an attractive alternative for the quantification of DNA in forensic samples. qPCR methods offer a number of potential advantages relative to the hybridization-based human-specific slot blot quantitations currently in use by most forensic labs. These advantages include: (1) minimization of hands-on lab time in obtaining data; (2) ease and speed of data interpretation for the analyst; (3) ability to quantify various genomes or chromosomes of forensic interest (e.g., human nuclear, Y, mitochondrial, etc.); (4) possibility of multiplexing two (or more) quantifications in a single tube, thus saving time and extracted DNA.

We have developed several human-specific assays for the quantification of DNA in forensic samples, in particular, for the human nuclear genome (TH01 target with TaqManâ detection), for the human mitochondrial genome (NDI target with TaqManMGBâ detection), and for the human Y-Chromosome (SRY target with TaqManMGBâ detection). In addition, a protocol has been developed to duplex the nuclear mitochondrial quantifications. These protocols will be described, and their applications will be demonstrated via the use of a laptop personal computer. Results from validation studies of the nuclear/mitochondrial duplex qPCR assay will be available. These results include investigations of sensitivity, accuracy, and species, specifically of the duplex assay, as well as quantifications of DNA extracted from a variety of sample types, including degraded DNA samples.

Application of a Single Robotics Platform to the Analysis of DNA in Forensic Samples

Pat W. Wojtkiewicz

The North Louisiana Criminalistics Laboratory (NLCL) has investigated the use of an automated platform, which will perform DNA purification, quantification, and amplification on forensic casework samples. The MWG RoboAmp® 4200 PE platform was designed to NLCL specifications to perform all three functions unattended and without cross-sample contamination. During initial research, the deck arrangement, pipetting, vacuum system, and plate handling were configured to optimize the sample processing flow. Additional software programs were integrated to further limit the analyst's work to three functions: initial sample processing; loading amplified product on gels; and final genotype analysis.

The three functions of the automated system have been set up as modules, so that samples may be started at several entry points in the sample-processing scheme. The modular setup also allows for new technologies to replace portions of the processing. Presently, PicoGreen, AluQuant, and Real-Time PCR can do the quantification. The final validation of AluQuant technology is waiting upon adding a illuminometer with sufficient sensitivity. Once validated, the AluQuant procedure can easily replace PicoGreen quantification.

Setting the real-time PCR on the ABI 7000 has also been developed and is currently being validated. The automated robotic system can use the typical forensic sample and set up the reaction plate for both human and Y-Chromosome quantification. The robotic system also sets up the standard curves.

Any type of sample can be processed through the system; however, the preferred samples are those that contain larger quantities of DNA and can be repeated, if necessary. The recovery efficiency as compared to organic extraction is approximately 50 percent. At this stage of

research, bloodstain hole-punches and buccal swabs have been tested to optimize recovery methods and verify reproducibility. The system can set up dilutions and amplify any sample that contains from 0.05 to 5.0 ng/µL DNA.

An automated platform that can perform the above functions benefits trained DNA analysts by relieving some of the repetitive work in DNA analysts, especially for samples that have adequate quantities of DNA. It also assists in setting up 96-well plates with less chance of getting samples mixed up.

Plenary Session, Tuesday, 2:00 pm – 3:30 pm

NIJ Programs, Funding Opportunities, and the DNA Assessment - Final Report

2004 Forensic DNA Legislative Update and the "Attorney Generals' Report on the DNA Evidence Backlog"

Tim Schellberg

During this talk, Mr. Schellberg will explain to NIJ grantees which states in 2004 passed DNA expansion legislation. He will also evaluate congressional activity taking place in 2004 and review particular DNA databasing legislative policy trends, with an emphasis on arrestee testing legislation. The legislative data indicates how larger databases have a dramatic effect on motivating additional casework.

The second half of Mr. Schellberg's presentation discusses findings of the "Attorney Generals Report on the DNA Evidence Backlog." In partnership with Washington State University, Mr. Schellberg's firm, Smith Alling Lane, designed and prepared the Attorney General's report. In addition to quantifying the size of the DNA backlog, Mr. Schellberg will discuss the report's findings on capacity issues, law enforcement perceptions, and how larger databases and use of casework prevent crime.

Plenary Session, Wednesday, 9:20 am - 10:20 am

Services and Support for Practitioners and Researchers

The Forensic Resource Network

Susan D. Narveson

The Forensic Resource Network (FRN) was created by the National Institute of Justice as a mechanism to increase the capabilities and capacities of forensic laboratories. The FRN includes: Marshall University Forensic Science Center, Huntington, West Virginia; the National Center for Forensic Science, Orlando, Florida; the National Forensic Science Technology Center, Largo, Florida; and West Virginia University Forensic Science Initiative. The FRN supports state and local crime laboratories in their efforts to establish and maintain the level of quality services demanded by the criminal justice system and the public. This is accomplished by providing innovative solutions to challenges facing the forensic science community through the delivery of technology based training tools, aid in systems support, and quality assurance products.

This presentation provides information on the FRN members and the products and services that they have developed for delivery to state and local forensic laboratories at no cost. FRN successes in meeting the technology and training needs of the community and the development of model programs that can be transferred to state and local crime laboratories will be highlighted.

Commercialization Assistance

F. Wayne Barte

The Office of Law Enforcement Technology Commercialization (OLETC) is a unique, federally funded resource whose primary mission is to assist innovators and entrepreneurs in the commercialization of new technology for use by the law enforcement and corrections (LEC) community.

Support for Procurement

Kevin Lothridge

This session will address how to deal with potential procurement problems. These include sole source agreements, blanket purchase agreements, (BPA) and quality and quantity of supplies. The tools and techniques discussed in this session will be useful for the forensic laboratory staff and procurement officers.

Plenary Session, Wednesday, 10:50 am – 11:30 am

Building Better Programs

The Importance of Practitioners to NIJ's DNA Grant Programs

John J. Behun

This presentation discusses the importance of involving the DNA practitioner in developing and managing NIJ's DNA grant programs, specifically those for backlog reduction and capacity enhancement. In order to be truly effective, and to assist in identifying potential problems, NIJ's DNA grant programs must incorporate lessons learned from the experience of DNA examiners, technicians, and laboratory managers. Advocating and justifying funding, measuring program and laboratory performance, and setting the parameters of program eligibility all benefit from the "real world" experience of the laboratory personnel who handle DNA cases in the laboratory every day. The presentation will demonstrate how NIJ is using practitioner input in its management of current DNA grants and will discuss ways in which practitioners can benefit from increased participation with NIJ in future DNA grant programs.

Building Better Programs: How Researchers and Practitioners Can Enhance NIJ Programs

Lois A. Tully

The goal of NIJ's Forensic DNA Research and Development Program is to harness the tremendous growth in the fields of molecular biology, genetics, and biotechnology by directing research toward the development of discriminatory, reliable, economical, and rapid DNA testing methods for forensic purposes. Through this research, NIJ endeavors to maximize the value of DNA evidence. Input from practitioners is critical for ensuring that NIJ continues to implement programs that best serve the forensic community. Examples of ways in which this can be accomplished include practitioner focus groups tasked with identifying areas that would benefit from further research, participation in the peer-review of research proposals submitted to NIJ for funding, collaborations with researchers, and participation in NIJ Program Review sessions.

Plenary Session, Wednesday, 1:20 pm – 2:20 pm

Programs of NIST and the FBI

New Assays for Improved Analysis of Degraded DNA and an Overview of the 2004 Interlaboratory Quantitation Study

John M. Butler, Margaret C. Kline, Peter M. Vallone, Jill E. Appleby, Michael D. Coble, Amy E. Decker, Janette W. Redman

NIST researchers are involved in a number of projects to support current DNA technologies and to develop new ones. A current focus at NIST is the development of new autosomal markers that can be amplified as small PCR products (50-100 bp) to improve the detection of degraded DNA samples. Reduced size single nucleotide polymorphism (SNP) and miniSTR markers are being evaluated for potential forensic usage. Results from analysis of 70 SNP markers and six new miniSTR markers (unlinked to the 13 CODIS loci) will be discussed in

terms of their variability in U.S. populations and the performance of these assays on degraded DNA material. In addition to their forensic utility for recovering information from degraded DNA, these new loci should prove useful for resolving complex paternity issues (e.g. incest) and identifying victims of mass disasters where insufficient family references are available.

NIST also conducts inter-laboratory studies to benefit the forensic DNA typing community. The most recent one, NIST Quantitation Study 2004, consisted of eight DNA extracts labeled A – H that were shipped to 84 laboratories representing 37 states and Puerto Rico, five foreign countries, eight private companies, two universities, and four federal laboratories. A total of 80 laboratories (95%) returned 287 sets of nuclear DNA quantitation data with an additional four sets of Y-Chromosome quantitation information. This study permitted a direct comparison of 20 different DNA quantitation assays within and between laboratories (and analysts) and was designed to assist in assessing the minimum stable DNA concentration to aid packaging of a future DNA Quantitation Standard Reference Material (SRM 2372).

R&D at the Counterterrorism and Forensic Science Research Unit of the FBI

Kerri Dugan

The Counterterrorism and Forensic Science Research Unit (CTFSRU) drives development and application of new technologies to provide technical advancements in the field of forensic science. The CTFSRU accomplishes this goal through both internal and outsourced R&D efforts. The biological sciences group within CTFSRU is responsible for over 50 R&D projects to advance human and microbial forensic genetic analyses, with a large part of the effort focused on automating the forensic analysis of human biological evidence. The forensic biology R&D effort consists of three main programs: human nuclear and mitochondrial DNA analysis, automation of STR analysis, and microbial forensics. Ongoing research and development within the biological sciences group to improve forensic biological analyses will be discussed.

An Update of CODIS and NDIS

Thomas F. Callaghan

The Combined DNA Index System (CODIS) software application that supports the National DNA Index System (NDIS), State DNA Index System (SDIS), and Local DNA Index System (LDIS) is now a stable product with a sizable customer base of over 200 domestic and international laboratories (175 domestic and 34 international in 21 countries) as well as 166 participating NDIS sites in 50 States. The deployment of the most recent version of CODIS software (CODIS 5.7) began in January 2004 and is expected to be completed by the end of June 2004.

The NDIS Policies and Procedures Board which has the responsibility of establishing, reviewing, and modifying the NDIS Policies and Procedures (operational procedures for participation in NDIS) was expanded at the end of last year to include additional state, local and FBI representatives. All of the state and local representative members of the NDIS Procedures Board are appointed by the Chief of the CODIS Unit. The FBI representatives are designated members by virtue of their positions. State and local representatives include: New York City Office of the Chief Medical Examiner, Florida Department of Law Enforcement, Kansas City Regional Crime Laboratory, Oregon State Police, and Illinois State Police. Two new procedures,

International Request for NDIS Search and Suspension of a Laboratory from NDIS, were recently approved by the NDIS Board.

To date, CODIS has aided over 17,000 investigations. In addition, there are over 1.7 million convicted offender and 87,000 forensic profiles in NDIS.